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# 3H-2-DG assay

Dongjoo You Evan D Rosen * Sona Kang *

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*For correspondence: erosen@bidmc.harvard.edu, kangs@berkeley.edu

An abbreviated version of this protocol was published in eLIFE in Nov 2017

Dnmt3a is an epigenetic mediator of adipose insulin resistance

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## Detailed protocol

**Glucose Uptake Assay**

**Solutions needed :**

1. KRH Buffer. pH is critical. Warm up at 37 C before starting the experiment.

| **KRH buffer** | Stock | 1X |
|---|---|---|
| KCl (74.56) | 1M | 5mM |
| MgSO4.7H2O (246.48) | 1M | 1.2mM |
| NaCl | 5M | 121mM |
| CaCl2 | 1M | 0.33mM |
| HEPES | 1M | 12mM |
| ddH2O, pH 7.4 | | |

2. KRH + Insulin (Final Concentration: 100nM of insulin in KRH).
3. Washing Buffer
200mM glucose in KRH buffer. (Add 10 ml 2M glucose solution and 90ml KRH Buffer).
4. Lysis buffer : 0.1% SDS Solution
5. 4 ml each of Ecolite solution in scintillation vials.
6. 2DG (2-Deoxy-D-glucose, 98%, FW 164.16), working solution: 100mM 2DG in water(16.416mg/ml)
7. Radioisotope : 2-deoxy-d-[2,6-3H]-glucose (0.33 mCi/ml)
 * from PerkinElmer NEN Radiochemicals

**Protocol:**

1. Wash cells with 250ul Serum free DMEM once, then add 200ul serum free DMEM
2. Place in incubator(37C) for 6 hours.
3. Wash cells with 250ul KRH Buffer once.
4. Stimulation for 20 minutes in incubator(37C) after adding the following accordingly
- Basal: Add 200ul KRH Buffer
- Insulin: Add 200ul 100nM Insulin in KRH Buffer
5. On the stimulation Buffer add 25 ul per well of radioisotope+2DG+KRH Buffer for both Basal and Insulin.
6. Place in incubator (37C) for 10 mins
7. Wash 3 times with 280ul per well of washing buffer which is KRH + 200mM Glucose
9. Lysis cells with 200ul of 0.1% SDS for 20 minutes.
10. Collect cells by pipetting up and down and add to scintillation vials which contain 4ml Ecolite solution. Save a small volume of lysates for protein quantification.

1. You, D. , Rosen, E. and Kang, S. (2021). 3H-2-DG assay. Bio-protocol Preprint. bio-protocol.org/prep1426.
2. You, D., Nilsson, E., Tenen, D. E., Lyubetskaya, A., Lo, J. C., Jiang, R., Deng, J., Dawes, B. A., Vaag, A., Ling, C., Rosen, E. D. and Kang, S. (2017). Dnmt3a is an epigenetic mediator of adipose insulin resistance. eLIFE. DOI: 10.7554/eLife.30766